Full text open access online (Since 2001)

PRINT: ISSN 0972-3757 ONLINE: ISSN 2456-6330
Int J Hum Genet, 24(2): 214-225 (2024)
DOI: 10.31901/24566322.2024/24.02.876

Role of Micro Ribonucleic Acid-124 in Protecting Against Nerve Injury After Cerebral Ischemia/Reperfusion

Li Wen[1], Liang Dang[1] and Ying Pu[2*]

1. Department of Neurology, Shandong Provincial Hospital Affiliated to Shandong First Medical University, 324 Jingwu Road, Jinan 250021, Shandong Province, China
2. Department of Function Test, Central Hospital Affiliated to Shandong First Medical University, Jinan 250013, Shandong Province, China

KEYWORDS Cerebral Ischemia. Micro Ribonucleic Acid-124. Nerve Injury. Rat. Reperfusion

ABSTRACT The researchers aimed to evaluate micro ribonucleic acid (miR)-124 for its protective role in nerve injury in rats following cerebral ischemia/reperfusion. A focal middle cerebral artery embolism model was constructed. Compared with sham operation group, model and miR-124 NC groups had significantly increased miR-124 expression, neurobehavioral score, malondialdehyde (MDA) content, brain infarct volume, and apoptosis rate, elevated expression levels of Bcl-2-associated X (Bax) protein as well as cleaved caspase-3, 8 and 9, attenuated activity of superoxide dismutase (SOD), and lowered expression of B-cell lymphoma 2 (Bcl-2) ($P<0.01$). By contrast to those in model and miR-124 NC groups, Bcl-2 expression rose, miR-124 expression, neurobehavioral score, brain infarct volume percentage and apoptosis rate reduced, Bax, cleaved caspase-3, 8 and 9 expression levels declined, SOD activity enhanced, and MDA content in miR-124 mimic group dropped ($P<0.01$). In conclusion, miR-124 protects rats from nerve injury after cerebral ischemia/reperfusion by improving the antioxidant capacity and regulating the expressions of apoptosis-associated proteins.

INTRODUCTION

Ischemic cerebrovascular disease has high incidence, disability and mortality rates, showing complex pathogenesis (Wang et al. 2022). Its subtypes include focal cerebral ischemia and global cerebral ischemia (Sharma et al. 2020). Focal cerebral ischemia is represented by a reduction or blockage of blood flow in the specific region of cerebral vessels, while global cerebral ischemia is clinically triggered by pulmonary embolism, shock and asphyxia. Blood reperfusion during ischemia initiates a series of endogenous injuries, thus aggravating cerebral ischemia, and this process is called cerebral ischemia/reperfusion injury (Tsaroucha et al. 2018; Mehrnoosh et al. 2019). This injury involves cerebrovascular microenvironment and numerous harmful factors such as oxygen free radicals released by nerve cells, which impairs the structure of cell biofilms and the function of cell mitochondria, thus ultimately resulting in apoptosis (Vongsfak et al. 2021). Hence, scavenging oxygen free radical products and suppressing apoptosis can protect nerve cells after cerebral ischemia/reperfusion injury (Wang et al. 2020).

*Address for correspondence:
Ying Pu
E-mail: puyingchasfmu@shu-edu.cn

Micro ribonucleic acids (miRNAs), a class of highly conservative small RNAs with about 18-25 nt in length, can block the translation of their target genes by binding their 3'-UTRs, thereby participating in modulating cell growth, differentiation, multiplication as well as apoptosis (Chen et al. 2022). Emphasis of previous research on miRNAs was mainly laid on tumorigenesis and immune diseases, but some recent studies have revealed the close relationship between miRNAs and ischemic cerebrovascular disease (Xu et al. 2022). For example, in the case of focal middle cerebral artery embolism (MCAO), Jeyaseelan et al. (2008) reported that 106 miRNAs were abnormally expressed in related rat model. MiR-124 exhibits a specific expression in the central nervous system, with its expression level in this system over 100 times that of other tissues (Zhang et al. 2023). As reported before (Liu et al. 2019), the depletion of the miR-124 expression in brain nerve cells is probably a subcellular indicator for cerebral ischemia/reperfusion injury. Besides, the knockout of miR-124 gene can induce strong innate immune activity to trigger neurogliocyte apoptosis (Xie et al. 2022). Doeppner et al. (2013) triggered the overexpression of miR-124 to evidently relieve focal cerebral ischemia, but its specific action mechanism still needs further exploration.

Objectives

This research was intended to assess miR-124 for its neuroprotective role in MCAO rats and to explore related mechanism.

METHODOLOGY

Animals

A total of 48 rats [Beijing Vital River Laboratory Animal Technology Co., Ltd., SCXK (Beijing) 2016-0011, China, Sprague-Dawley, male, SFP-grade, weight: (200±20) g] were selected. They were kept at the temperature of (23±2)°C and provided with free water plus food.

Main Reagents Together With Apparatus

Beyotime Biotechnology Institute (China) was the supplier of mouse monoclonal antibody against glyceraldehyde 3-phosphate dehydrogenase, bicinchoninic acid protein quantitative kit, as well as annexin V-fluorescein isothiocyanate apoptosis detection kit. Nanjing KeyGen Biotech Co., Ltd. (China) supplied the rabbit polyclonal antibodies against B-cell lymphoma 2 (Bcl-2), Bak1, and Bcl-2-associated X protein (Bax) (Abcam, USA), in combination with activity detection kits for caspase-3, -9, and -8 . The kits for examining superoxide dismutase (SOD) activity plus malondialdehyde (MDA) content manufactured by Nanjing Jiancheng Bioengineering Institute (China) were employed. MiR-124 mimic (UAACCGATTTCAAAUGGUGCUA) and miR-124 negative control (miR-124 NC, CAGTACUUUUGUGUAGUACAAA) sourced from Shanghai GenePharma Co., Ltd. (China). Electrophoresis-related instruments together with gel imaging system were offered by Bio-Rad (USA). Microplate reader of TECAN Group (Switzerland) was employed. Flow cytometer was obtained from BD (USA).

Grouping and Administration

Sham operation, miR-124 NC, model, and miR-124 mimic groups were set up to recruit 48 rats in total. Artificial cerebrospinal fluid was utilized to dissolve miR-124 NC and miR-124 mimic at 20 ìmol/L in concentration, followed by injection of the obtained solution (a total of 5 ìL) into the lateral ventricle at an injection rate of 1 ìL/min. Artificial cerebrospinal fluid was also injected into sham operation and model groups 24 h after the model was established (Huang et al. 2015).

Modeling

With reference to a relevant literature (Ansari et al. 2011), the MCAO model was established. Specifically, after anesthesia using chloral hydrate (10%), the rats were laid in the supine position. Then the neck skin was cut in the middle to bluntly dissect the muscles on its both sides. Furthermore, the separation of right common carotid artery (CCA), right external carotid artery (ECA), and right internal carotid artery (ICA) was accomplished, sutures were applied to ligate ECA together with CCA, and a vascular clip was employed to clamp ICA from the distal end. Meanwhile, ECA near CCA was cut open to make a small incision, the ICA clip was released, and ECA was slowly implanted with the blunt nylon thread by a depth of about 18 mm, resulting in cerebral ischemia. In the end, the skin was disinfected and sutured, with the nylon thread left outside by about 1 cm. At 2 h after ischemia, resistance was put on the nylon thread by gentle lifting to restore the blood flow, thereby realizing 22 h of reperfusion. Only ECA, CCA and ICA underwent isolation in sham operation group, without CCA ligation.

Neurobehavioral Scoring

Following 24 h of operation, the neurobehavioral function was scored based on the Zea Longa 5-point scale as follows: no symptoms of nerve injury rated as 0 points, inability of the left forelimb to fully contract scored 1 point, rotation to the left during walking valued as 2 points, tilt to the left when walking graded as 3 points, and failure to spontaneously walk and loss of consciousness assessed at 4 points.

Measurement of Brain Infarct Volume

The rats were anesthetized at 24 h subsequent to operation and then quickly decapitated, with the cerebellum and olfactory bulb of the brain tissues removed. Then the whole brain was

acquired to prepare sections (thickness: 2 mm), immediately followed by soaking with triphenyltetrazolium chloride (2%) and staining for 30 min in a dark incubator at 37°C. White infarcted brain tissues along with red normal brain tissues were observed. Next, the tissue slices were fixed in 10% formaldehyde and photographed. Later, the Image-Pro Plus 6.0 software from Media Cybernetics, Inc. (USA) was adopted for the calculation of brain infarct volume, followed by multiplication of the obtained result by 2 mm (the brain slice thickness). Afterwards, the totaled brain infarct volume of all brain slices was the cerebral infarction volume of the whole brain, and the brain infarct volume percentage (BIVP) was calculated.

Detection of the Expression of miR-124 by Reverse Transcription-Polymerase Chain Reaction (RT-PCR)

TRIzol method was carried out for obtaining total RNA that was subjected to one-step RT-PCR, and the RT products underwent gel electrophoresis. Shanghai Sangon Biotech Co., Ltd. (China) was responsible for the synthesis of the primer of miR-124 (5'-UAGCACCAUUUGAAA UGGUUA-3'). RT-PCR was performed on the RT system consisting of total RNA templates [2 μL (1 μg)], dNTP mixtures (2 μL), $MgCl_2$ (2 μL) and DEPC-treated distilled water (25 μL), with conditions set as 5 min of 95°C pre-denaturation, prior to 30-s denaturation (95°C), 58°C annealing for 30 s and 30 s of 72°C extension in 40 cycles.

Examination of MDA Content Plus SOD Activity

After homogenization of the ischemic brain tissues, MDA content was detected together with SOD activity *as per* the instructions of corresponding kits.

Detection of Apoptosis of Ischemic Brain Cells by Flow Cytometry

After homogenization, the ischemic brain tissues were digested with trypsin and collagenase to obtain cell suspension. Subsequently, the ischemic brain cells were inspected *via* the flow cytometer to obtain their apoptosis with annexin V-fluorescein isothiocyanate kit within 1 h.

Measurement of Cleaved Caspase-3, Bcl-2, Cleaved Caspase-8, Bax, and Cleaved Caspase-9 Proteins

Western blotting was adopted for detection. In detail, the ischemic brain tissues were homogenized, followed by lysis with RIPA lysis buffer (strong) and centrifugation, and the proteins were finally harvested. Next, the bicinchoninic acid kit was employed to determine the protein concentration. Following denaturation and loading, the proteins underwent sodium dodecyl sulfonate gel electrophoresis and membrane transfer. Later, primary antibodies were supplemented for overnight (4°C) culture of the proteins, before room-temperature culture (1-2 h) with secondary antibodies. With the gel imaging system, protein exposure was performed, and each protein band was analyzed by virtue of Quantity One software (Bio-Rad, USA) for the gray-scale value.

Statistical Analysis

Statistical analysis was implemented by means of SPSS 26.0 software (IBM Inc., USA). After the homogeneity of variance and normal distribution examinations of all data, mean ± standard deviation was selected to express the normally distributed measurement data, with the *t*-test conducted for intergroup comparisons. The statistically significant differences were denoted by $P<0.05$.

RESULTS

Role of miR-124 Mimic in Affecting miR-124 Expression in Brain Tissues

Model group and miR-124 NC group, compared with sham operation group, presented a markedly raised miR-124 expression in rat brain tissues ($P<0.05$). By contrast to those in model and miR-124 NC groups, miR-124 in the miR-124 mimic group rose remarkably in terms of the expression level ($P<0.05$) (Fig. 1).

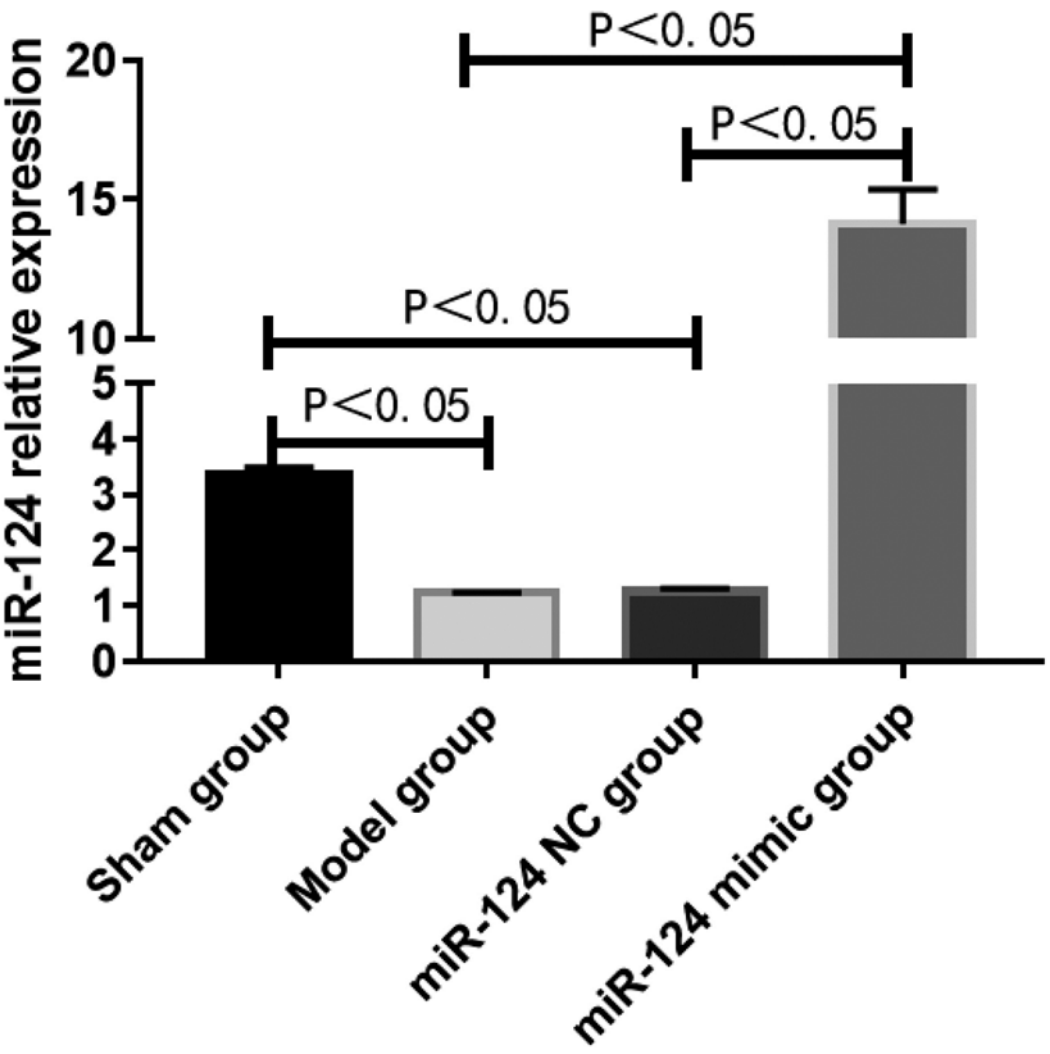

Fig. 1. Role of miR-124 mimic in affecting miR-124 in brain tissues at the expression level
Source: Author/s

Function of miR-124 Mimic in Influencing Neurobehavioral Score

In contrast to the sham operation group, model and miR-124 NC groups had markedly elevated neurobehavioral scores (P<0.05). Besides, the neurobehavioral score notably dropped in miR-124 mimic group by contrast to those of model and miR-124 NC groups (P<0.05) (Fig. 2).

Effect of miR-124 Mimic on Brain Infarct Volume

Compared to the sham operation group, model and miR-124 NC groups had obviously increased BIVP (P<0.05). Moreover, miR-124 mimic group exhibited remarkably lower BIVP than model and miR-124 NC groups (P<0.05) (Fig. 3).

Impacts of miR-124 Mimic on Brain Tissues Regarding MDA Content Besides SOD Activity

By contrast to sham operation group, model and miR-124 NC groups displayed raised MDA content in addition to weakened SOD activity (P<0.05). Furthermore, the SOD activity was enhanced, and the MDA content was reduced in the miR-124 mimic group contrasting with miR-124 NC and model groups (P<0.05) (Fig. 4).

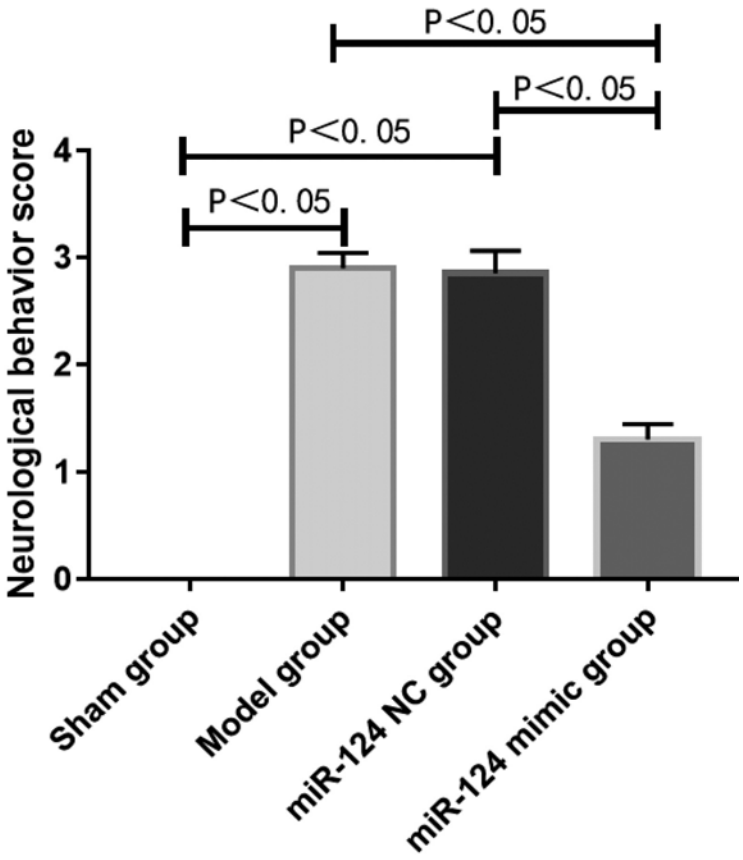

Fig. 2. Function of miR-124 mimic for influencing neurobehavioral score
Source: Author/s

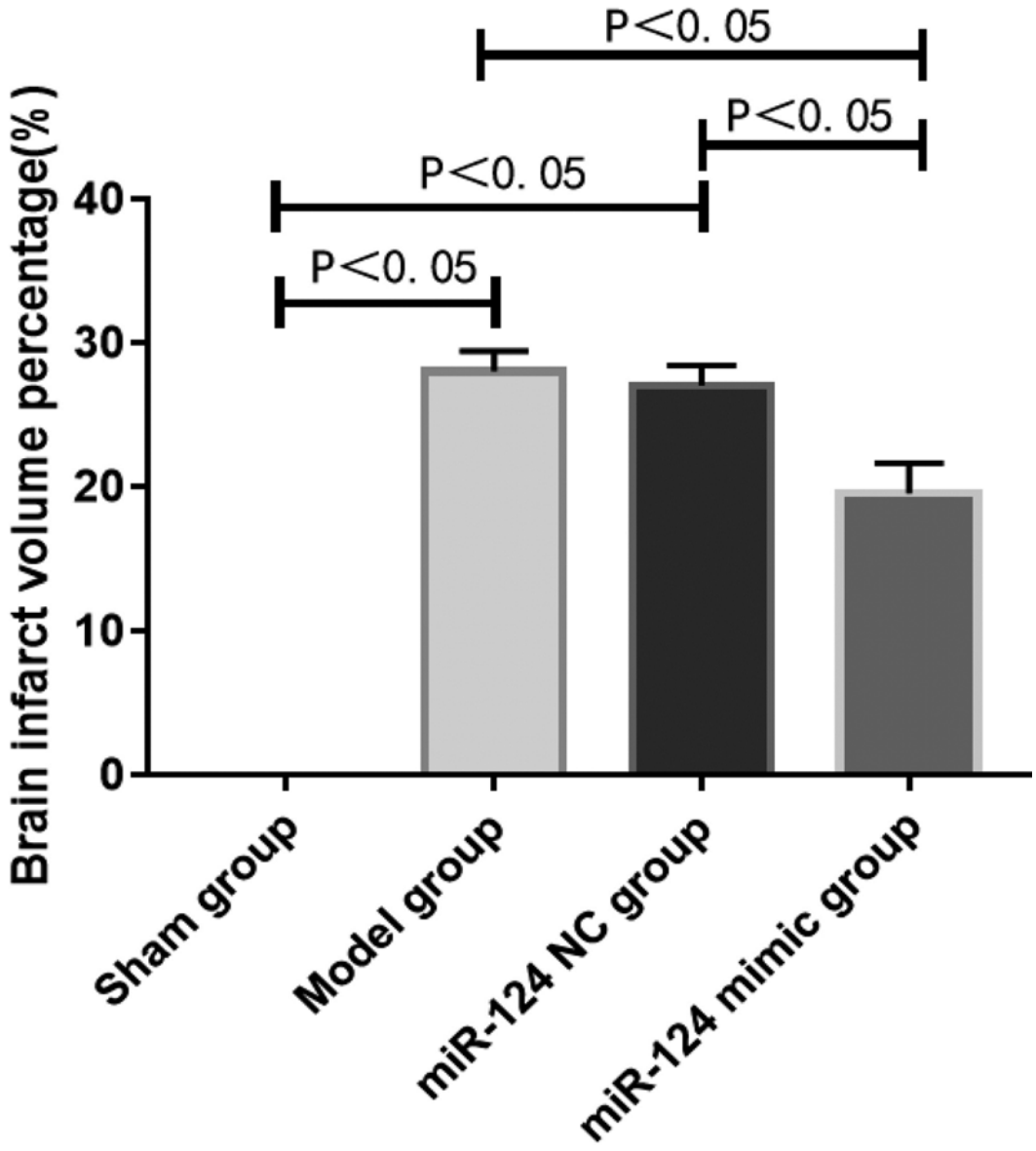

Fig. 3. Role of miR-124 mimic in impacting brain infarct volume
Source: Author/s

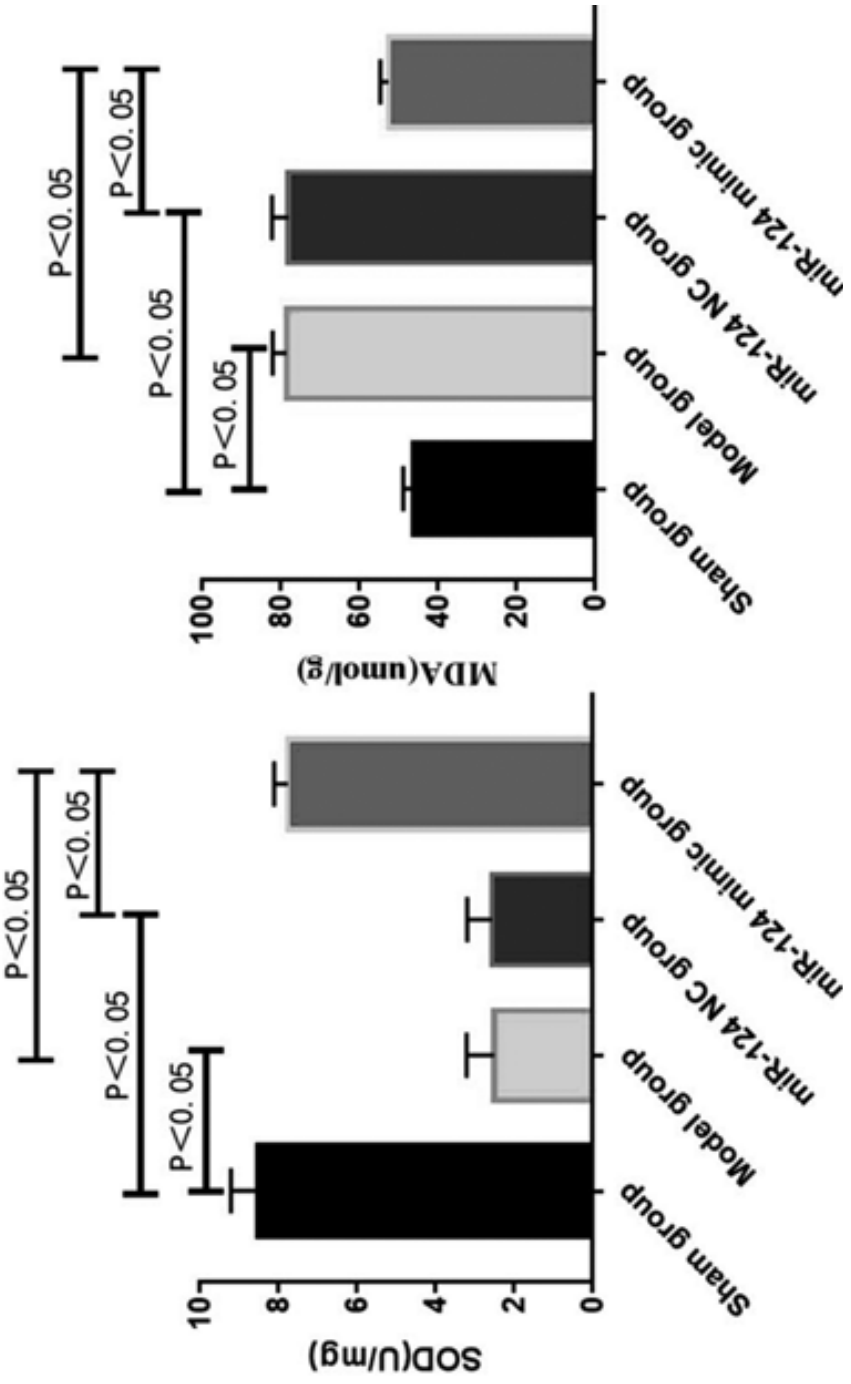

Fig. 4. Effects of miR-124 mimic on brain tissues regarding MDA content plus SOD activity
Source: Author/s

Role of miR-124 Mimic in Influencing Ischemic Brain Cell Apoptosis

In contrast to the sham operation group (1.98±0.23), the apoptosis rate showed a markedly elevation in the model group (9.21±1.02) and the miR-124 NC group (9.15±1.13) (P<0.05). Additionally, the significantly decreased apoptosis rate occurred in miR-124 mimic group (4.38±0.42) in contrast to those of model and miR-124 NC groups (P<0.05) (Fig. 5).

Functions of miR-124 Mimic for Affecting Cleaved Caspase-8, 3 and -9 Regarding Their Protein Expressions in Brain Tissues

As shown in Figure 6, by contrast to sham operation group, miR-124 NC plus model groups manifested markedly increased cleaved caspase-9, -3 and -8 at the protein expression level (P<0.05). Compared to those in model and miR-124 NC groups, cleaved caspase-3, -8 and -9 presented reduced protein expression levels in the miR-124 mimic group (Fig. 6).

Impacts of miR-124 Mimic on Bax and Bcl-2 Protein Expressions in Brain Tissues

As for the protein expression levels, through contrasting with sham operation group, model and miR-124 NC groups were detected with notable up-regulation of Bax and dramatic down-regulation of Bcl-2 P<0.05). A notably lower Bax protein expression level but an obviously higher Bcl-2 protein expression level than those in model and miR-124 NC groups were measured (P<0.05) (Fig. 7).

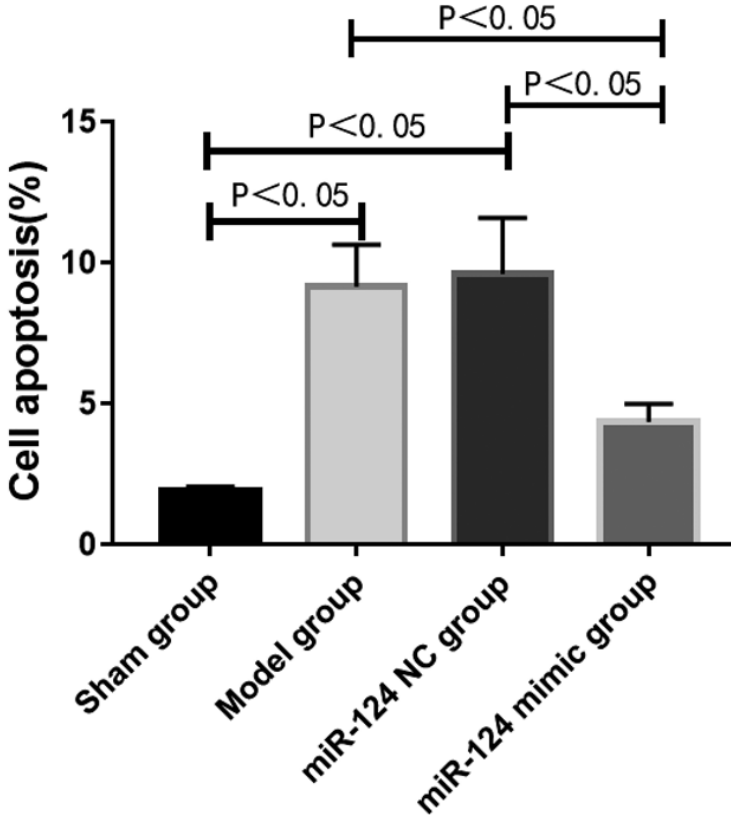

Fig. 5. Mechanism of miR-124 mimic in influencing ischemic brain cell apoptosis
Source: Author/s

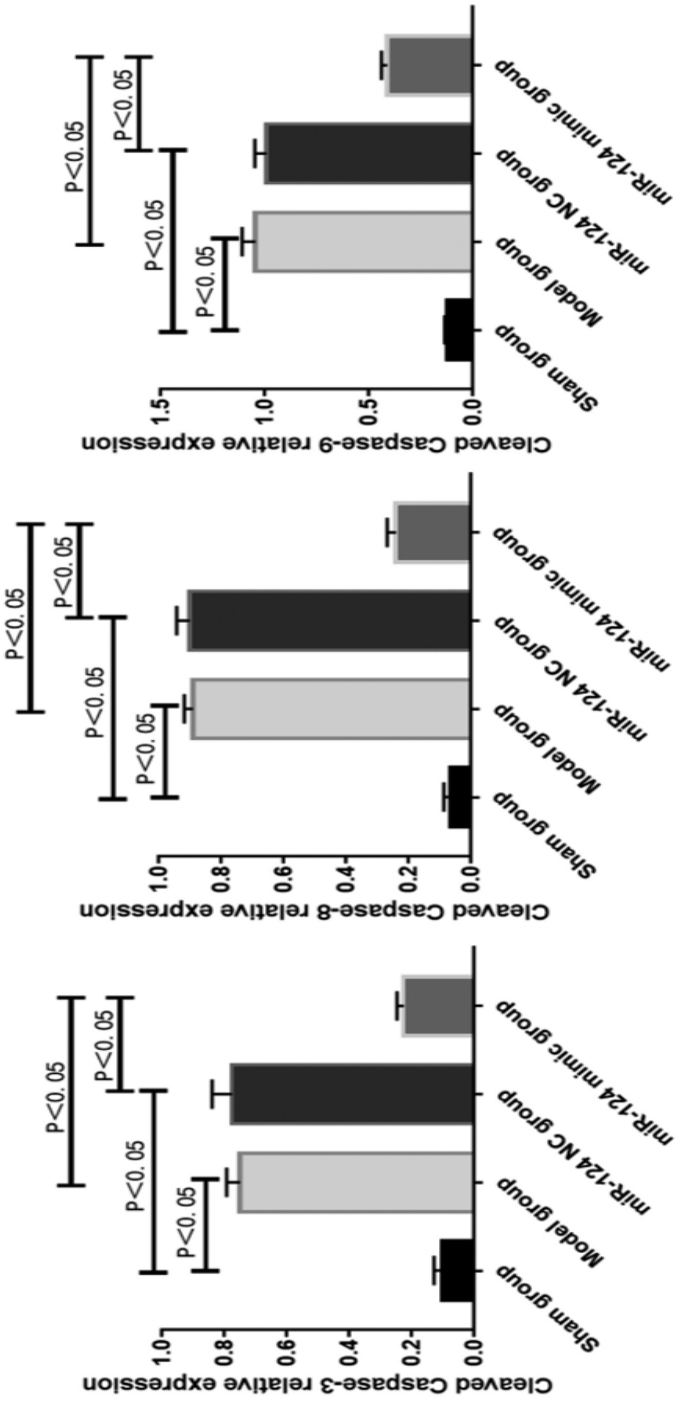

Fig. 6. Impacts of miR-124 mimic on cleaved caspase-3, -9 & -8 at the protein expression level in brain tissues of MCAO
Source: Author/s

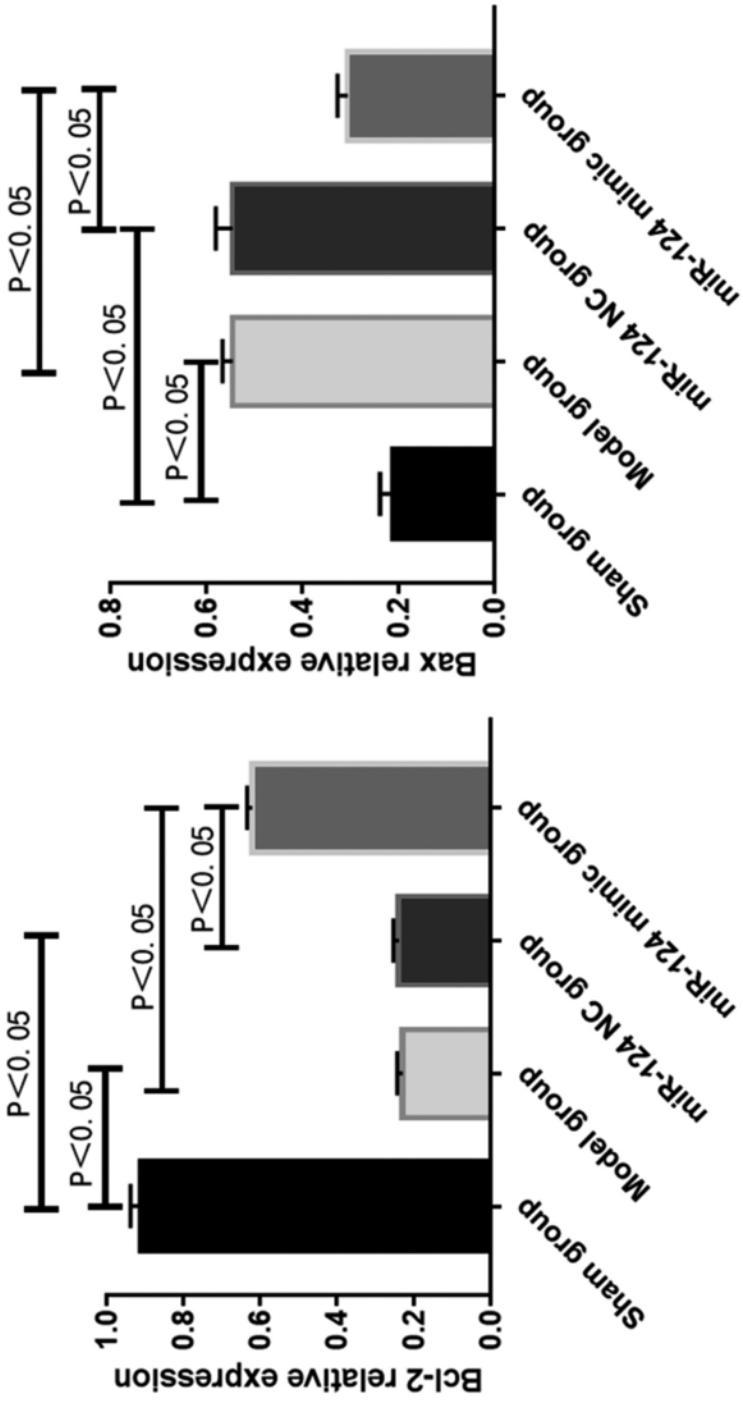

Fig. 7. Impacts of miR-124 mimic on the protein expressions of Bax and Bcl-2 in the brain tissues of MCAO rats
Source: Author/s

DISCUSSION

The restoring of blood supply after cerebral ischemia triggers the production of numerous damaging factors, such as oxygen free radicals, in the microenvironment of brain vessels (Li et al. 2022). Hence, searching for an effective substance for scavenging oxygen free radicals and then resisting cell injury are crucial for the neuroprotection after cerebral ischemia/reperfusion injury (Abed et al. 2020).

MiR-124 has a neuroprotective effect on ischemic brain injury (Xiong et al. 2020). Consistently, miR-124 mimic in this research prominently reduced the neurobehavioral score and brain infarct volume of rats and suppressed apoptosis. MDA is a by-product of cell membrane lipids oxidized by reactive oxygen species, and a biomarker indicating oxidative stress-induced cellular damage. Being the main antioxidant enzyme in cells, SOD helps scavenge oxygen free radicals and resists the damage of oxygen free radicals to cells (Yu et al. 2019; Zhao et al. 2020). As revealed by the present study, miR-124 mimic markedly enhanced the SOD activity and reduced the MDA content, suggesting that miR-124 mimic cleared the oxidative stress products produced by cerebral ischemia-reperfusion injury and improved the activity of antioxidant enzymes (Forouzanfar et al. 2019).

The apoptosis of brain nerve cells is probably associated with the imbalance between anti- and pro-apoptotic genes with respect to their expressions in tissues (Chen et al. 2020; Tang et al. 2020). As the most common anti-apoptotic gene, Bcl-2 can generate a heterodimer with Bax as the pro-apoptotic gene, or Bax itself is capable of forming a dimer, for the purpose of modulating cell apoptosis (Moldoveanu and Czabotar 2020). Bak1 belongs to the Bcl-2 family as a pro-apoptotic protein, and the compound of Bak1 and Bax can maintain the stability of mitochondrial membranes. When the upstream apoptotic signal is transmitted, Bak1 can mediate the mitochondrial permeability transition pore to open for cytochrome C release in mitochondria and induce caspase signaling cascade reaction, finally leading to apoptosis (Singh et al. 2019).

Stimulated by the upstream apoptotic signal, caspase-8 and caspase-9, the initiator caspases, are activated by cleavage through other kinases or themselves to transmit the apoptotic cascade signal to caspase-3, an executor in the caspase family, thus ultimately inducing apoptosis (Aral et al. 2019). In this study, miR-124 mimic triggered apparent elevation of Bcl-2 in addition to decline of cleaved caspase-3, -8 and -9 at the protein expression level. Thus, miR-124 mimic can suppress the activation of caspase-3 through controlling apoptosis-related protein expressions and simultaneously blocking the endogenous and exogenous pro-apoptotic pathways of cells, thus inhibiting the apoptosis of brain cells induced by cerebral ischemia-reperfusion injury (Che et al. 2019).

CONCLUSION

In conclusion, miR-124 serves as a neuroprotector for rats in the case of cerebral ischemia/reperfusion injury *via* improving the antioxidant capacity while regulating apoptosis-related proteins from the aspect of expression.

RECOMMENDATIONS

This research paves the way for treating cerebral ischemia/reperfusion injury, but further clinical investigations are still in need.

ACKNOWLEDGMENTS

This study was not financially supported.

REFERENCES

Abed MN, Alassaf FA, Jasim MH et al. 2020. Comparison of antioxidant effects of the proton pump-inhibiting drugs omeprazole, esomeprazole, lansoprazole, pantoprazole, and rabeprazole. *Pharmacology*, 105(11-12): 645-651. doi: 10.1159/000506232

Ansari S, Azari H, McConnell DJ et al. 2011. Intraluminal Middle Cerebral Artery Occlusion (MCAO) Model for ischemic stroke with laser doppler flowmetry guidance in mice. *J Vis Exp*, 51: 2879. doi: 10.3791/2879

Aral K, Aral CA, Kapila Y 2019. The role of caspase-8, caspase-9, and apoptosis inducing factor in periodontal disease. *J Periodontol*, 90: 288-294. doi: 10.1002/JPER.17-0716

Che QQ, Huang T, Zhang YD et al. 2019. Effect of miR-124 on neuronal apoptosis in rats with cerebral infarction through Wnt/β-catenin signaling pathway. *Eur Rev Med Pharmacol Sci*, 23: 6657-6664. doi: 10.26355/eurrev_201908_18556

Chen FS, Tong XY, Fang B et al. 2022. The roles of microRNAs in spinal cord ischemia-reperfusion injury. *Neur Regen Res*, 17: 2593-2599. doi: 10.4103/1673-5374.339471

Chen Y, Shen J, Ma C et al. 2020. Skin-derived precursor Schwann cells protect SH-SY5Y cells against 6-OHDA-induced neurotoxicity by PI3K/AKT/Bcl-2 pathway. *Brain Res Bull*, 161: 84-93. doi: 10.1016/j.brainresbull.2020.03.020

Doeppner TR, Doehring M, Bretschneider E et al. 2013. MicroRNA-124 protects against focal cerebral ischemia via mechanisms involving Usp14-dependent REST degradation. *Acta Neuropathol*, 126(2): 251-265. doi: 10.1007/s00401-013-1142-5

Forouzanfar F, Shojapour M, Asgharzade S et al. 2019. Causes and consequences of microRNA dysregulation following cerebral ischemia-reperfusion injury. *CNS Neurol Disord Drug Targets*, 18: 212-221. doi: 0.2174/1871527318666190204104629

Huang LG, Li JP, Pang XM et al. 2015. MicroRNA-29c Correlates with neuroprotection induced by FNS by targeting both Birc2 and Bak1 in rat brain after stroke. *CNS Neurosci Ther*, 21(6): 496-503. doi: 10.1111/cns.12383

Jeyaseelan K, Lim KY, Armugam A 2008. MicroRNA expression in the blood and brain of rats subjected to transient focal ischemia by middle cerebral artery occlusion. *Stroke*, 39(3): 959-966. doi: 10.1161/STROKEAHA.107.500736

Li M, Tang H, Li Z et al. 2022. Emerging treatment strategies for cerebral ischemia-reperfusion injury. *Neuroscience*, 507: 112-124. doi: 10.1016/j.neuroscience.2022.10.020

Liu X, Feng Z, Du L et al. 2019. The potential role of MicroRNA-124 in cerebral ischemia injury. *Int J Mol Sci*, 21: 120. doi: 10.3390/ijms21010120

Mehrnoosh Sedighi, Sewell Robert DE, Nazari A et al. 2019. A review of the most important medicinal plants effective on cardiac ischemia-reperfusion injury. *Curr Pharm Des*, 25(3): 352-358. doi: 10.2174/1381612825666190329144016

Moldoveanu T, Czabotar PE 2020. BAX, BAK, and BOK: A coming of age for the BCL-2 family effector proteins. *Cold Spring Harbor Perspect Biol*, 12: a036319. doi: 10.1101/cshperspect.a036319

Tsaroucha AK, Valsami G, Kostomitsopoulos N et al. 2018. Silibinin effect on Fas/FasL, HMGB1, and CD45 expressions in a Rat Model subjected to liver ischemia-reperfusion injury. *J Invest Surg*, 31(6): 491-502. doi: 10.1080/08941939.2017.1360416

Sharma D, Maslov LN, Singh N et al. 2020. Remote ischemic preconditioning-induced neuroprotection in cerebral ischemia-reperfusion injury: Preclinical evidence and mechanisms. *Eur J Pharmacol*, 883: 173380. doi: 10.1016/j.ejphar.2020.173380

Singh R, Letai A, Sarosiek K 2019. Regulation of apoptosis in health and disease: the balancing act of BCL-2 family proteins. *Nat Rev Mol Cell Biol*, 20: 175-193. doi: 10.1038/s41580-018-0089-8

Tang H, Gamdzyk M, Huang L et al. 2020. Delayed recanalization after MCAO ameliorates ischemic stroke by inhibiting apoptosis via HGF/c-Met/STAT3/Bcl-2 pathway in rats. *Exp Neurol*, 330(4): 113359. doi: 10.1016/j.expneurol.2020.113359

Vongsfak J, Pratchayasakul W, Apaijai N et al. 2021. The alterations in mitochondrial dynamics following cerebral ischemia/reperfusion injury. *Antioxidants*, 10: 1384. doi: 10.3390/antiox10091384

Wang L, Xiong X, Zhang X et al. 2020. Sodium Tanshinone IIA Sulfonate Protects Against Cerebral Ischemia-reperfusion Injury by Inhibiting Autophagy and Inflammation. *Neuroscience*, 441: 46-57. doi: 10.1016/j.neuroscience.2020.05.054

Wang Y, Chen W, Zhou J et al. 2022. Nitrate metabolism and ischemic cerebrovascular disease: A narrative review. *Front Neurol*, 13: 735181. doi: 10.3389/fneur.2022.735181

Xie HM, Su X, Zhang FY et al. 2022. Profile of the RNA in exosomes from astrocytes and microglia using deep sequencing: implications for neurodegeneration mechanisms. *Neur Regen Res*, 17: 608-617. doi: 10.4103/1673-5374.320999

Xiong L, Zhou H, Zhao Q et al. 2020. Overexpression of miR-124 protects against neurological dysfunction induced by neonatal hypoxic–ischemic brain injury. *Cell Mol Neurobiol*, 40: 737-750. doi: 10.1007/s10571-019-00769-2

Xu Y, Hu Y, Xu S et al. 2022. Exosomal microRNAs as potential biomarkers and therapeutic agents for acute ischemic stroke: New expectations. *Front Neurol*, 12: 747380. doi: 10.3389/fneur.2021. 747380

Yu H, Zhang J, Ji Q et al. 2019. Melatonin alleviates aluminium chloride-induced immunotoxicity by inhibiting oxidative stress and apoptosis associated with the activation of Nrf2 signaling pathway. *Ecotoxicol Environ Saf*, 173: 131-141. doi: 10.1016/j.ecoenv.2019.01.095

Zhang WH, Jiang L, Li M et al. 2023. MicroRNA 124: An emerging therapeutic target in central nervous system disorders. *Exp Brain Res*, 241: 1215-1226. doi: 10.1007/s00221-022-06524-2

Zhao F, Cao F, Li H et al. 2020. The effects of a short-term exposure to propiconazole in zebrafish (Danio rerio) embryos. *Environ Sci Pollut Res Int*, 27(30): 38212-38220. doi: 10.1007/s11356-020-09968-7

Paper received for publication in April, 2023
Paper accepted for publication in February, 2024